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FORM PTO-1390 (Modified)(REV 10-94) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
7676-46 (971019AMSG)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 C.F.R. 1.5)
Not Yet Assigned 08/849543

Rec'd PCT/PTO 10 JUN 1997

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU95/00875	**22 December 1995 (22.12.95)**	**22 December 1994 (22.12.94)**

TITLE OF INVENTION: **Karim Rouan Cham**

APPLICANT(S) FOR DO/EO/US: **A TREATMENT FOR CARDIOVASCULAR AND RELATED DISEASES**

APPLICANT HEREWITH SUBMITS TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) THE FOLLOWING ITEMS AND OTHER INFORMATION:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [ ] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.

8. [ ] A translation of the amendments to the claims under PCT Article 19(35 U.S.C. 371(c)(3)).

9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (Executed)

10 [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). It is requested the examination be based on:
   a. [ ] the claims as originally filed.
   b. [ ] the claims as amended under PCT Article 19.
   c. [ ] the claims as annexed to the International Preliminary Examination Report.
   d. [ ] the claims as amended by the enclosed Preliminary Amendment.

ITEMS 11. TO 21. BELOW CONCERN DOCUMENT(S) OR INFORMATION INCLUDED:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [X] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.

14. [ ] A substitute specification.

15. [ ] A change of power of attorney and/or address letter.

16. [X] A Verified Statement Claiming Small Entity Status.

17. [X] A copy of the cover sheet of the PCT publication of the application (WO 96/19250).

18. [X] A copy of the International Preliminary Examination Report.

19. [ ] An English translation of the International Preliminary Examination Report.

20. [ ] Formal drawings (sheets).

21 [ ] Other items or information:

U.S. APPLICATION NO. (If known, see 37 CFR 1 5) **Not Yet Assigned**	INTERNATIONAL APPLICATION NO. **PCT/AU95/00875**	ATTORNEY'S DOCKET NUMBER **7676-46 (971019AMSG)**

	CALCULATIONS PTO USE ONLY	
22. [X] The following fees are submitted (enter lowest fee applicable):		
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO $910.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $700.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $770.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1,040.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$910.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 or [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	**$**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	22 - 20 =	2	x $ 22.00 =	**$44.00**	
Independent claims	1 - 3 =	0	x $ 80.00 =	**$ - 0 -**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00 =	**$**	
			TOTAL OF ABOVE CALCULATIONS =	**$954.00**	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				**$477.00**	
			SUBTOTAL =	**$477.00**	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 or [ ] 30 months from the earliest claimed priority date (37 CFR 1 492(f)) +				**$**	
			TOTAL NATIONAL FEE =	**$477.00**	
			TOTAL FEES ENCLOSED =	**$**	
				Amount to be: Refunded	**$**
				Charged	**$**

a. [ ] A check in the amount of $____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 16-0235 in the amount of $ 477.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 16-0235. A duplicate copy of this sheet is enclosed.

NOTE: WHERE AN APPROPRIATE TIME LIMIT UNDER 37 CFR 1.494 OR 1.495 HAS NOT BEEN MET, A PETITION TO REVIVE (37 CFR 1.137(a) OR (b)) MUST BE FILED AND GRANTED TO RESTORE THE APPLICATION TO PENDING STATUS.

SEND ALL CORRESPONDENCE TO:

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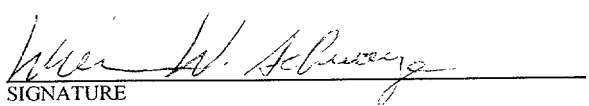
June 10, 1997
DATE


SIGNATURE

William W. Schwarze
NAME

25,918
REGISTRATION NUMBER

Express Mail Mailing Label No. EM508475774US

BOX PCT

IN THE UNITED STATES RECEIVING OFFICE

In re:	Patent Application of Karim Rouan Cham	: Group Art Unit: : Not Yet Assigned
International Application No.:	PCT/AU95/00875	: Examiner: Not Yet : Assigned
International Filing Date:	22 December 1995 (22.12.95)	:
U.S. Appln. No.:	Not Yet Assigned	:
Filed:	Herewith	:
For:	A TREATMENT FOR CARDIOVASCULAR AND RELATED DISEASES	: Attorney Docket : No. 7676-46 : (971019AMSG)

PRELIMINARY AMENDMENT

Simultaneously with the filing of the above-identified application with which this Preliminary Amendment is being filed, and prior to the calculation of the filing fee, Applicant hereby amends the application as follows:

In The Specification:

At page 20, before claim1, insert --I claim:--; and

After page 23, insert the Abstract from the cover page of WO96/19250, and change the heading to --ABSTRACT OF THE DISCLOSURE--.

In The Claims:

In claim 4, line 1, delete "Claim 2 or Claim 3" and insert -- Claim 2 --;

In claim 5, line 1, delete "any one of Claims 1 to 4" and insert -- Claim 1 --;

In claim 11, line 1 and 2, delete "any one of Claims 8 to 10" and insert -- Claim 8 --;

In claim 12, line 1 and 2, delete "any one of Claims 8 to 11" and insert -- Claim 8 --;

In claim 13, line 2, delete "any one of Claims 8 to 12" and insert -- Claim 8 --;

In claim 15, line 5, delete "any one of Claims 1 to 12" and insert -- Claim 1 --;

In claim 16, lines 4 and 5, delete "any one of claims 1 to 12" and insert -- Claim 1--;

In claim 17, lines 4 and 5, delete "any one of Claims 1 to 12" and insert -- Claim 1 --;

In claim 18, lines 4 and 5, delete "any one of Claims 1 to 12" and insert -- Claim 1 --;

In claim 19, lines 5 and 6, delete "any one of Claims 1 to 12" and insert -- Claim 1 --;

In claim 20, line 6, delete "any one of Claims 1 to 12" and insert -- Claim 1 --;

In claim 21, line 6, delete "any one of Claims 1 to 12" and insert -- Claim 1 --; and

In claim 22, lines 5 and 6, delete "any one of Claims 1 to 12" and insert -- Claim 1 --.

REMARKS

The purpose of this amendment is to insert the U.S. application heading and to eliminate the multiple dependent claims in this application. Entry of this amendment and early examination of this application are respectfully solicited.

Respectfully submitted,

KARIM ROUAN CHAM

June 10, 1997
Date

By ____________________
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WWS/md

TITLE

TREATMENT FOR CARDIOVASCULAR AND RELATED DISEASES

TECHNICAL FIELD

THIS INVENTION relates to plasma or serum delipidation in animals (which term shall indicate humans), to a treatment for cardiovascular disease and to removal of excess fat from the animals. In particular, it is directed to the removal of cholesterol, triglycerides and other lipids, and fat soluble toxins - for example, insecticides - from the blood plasma or serum of such animals.

BACKGROUND ART

Cardiovascular diseases are responsible for a significant number of deaths in most industrialised countries.

One such disease is atherosclerosis which is characterised by local fatty thickening in the inner aspects of large vessels supplying blood to the heart, brain and other vital organs. These lesions obstruct the lumen of the vessel and result in ischaemia of the tissue supplied by the vessel. Prolonged or sudden ischaemia may result in a clinical heart attack or stroke from which the patient may or may not recover.

The relationship between dietary lipid, serum cholesterol and atherosclerosis has long been recognised. In many epidemiological studies it has been shown that a single measurement of serum cholesterol has proved to be a significant predictor of the occurrence of coronary heart disease.

Thus diet is the basic element of all therapy for hyperlipidaemia (excessive amount of fat in plasma). However, the use of diet as a primary mode of therapy requires a major effort on the part of physicians, nutritionists, dietitians and other health professionals.

If dietary modification is unsuccessful, drug therapy is an alternative. Several drugs, used singly or in combination, are available. However, there is no direct evidence that any cholesterol-lowering drug can be safely administered over an extended period.

A combination of both drug and diet may be required to reduce the concentration of plasma lipids. Hypolipidaemic drugs are therefore used as a supplement to dietary control.

Many drugs are effective in reducing blood lipids, but none work in all types of hyperlipidaemia and they all have undesirable side effects. There is no conclusive evidence that hypolipidaemic drugs can cause regression of atherosclerosis. Thus, despite progress in achieving the lowering of plasma cholesterol to prevent heart disease by diet, drug therapies, surgical revascularization procedures and angioplasty, atherosclerosis remains the major cause of death in Western Countries.

In view of the above, new approaches have been sought to reduce the amount of lipid in the plasma of homozygotes and that of heterozygotes for whom oral drugs are not effective.

Plasmapheresis (plasma exchange) therapy has been developed and involves replacement of the patient's plasma with donor plasma or more usually a plasma

protein fraction. This treatment can result in complications due to the possible introduction of foreign proteins and transmission of infectious diseases. Further, plasma exchange removes all the plasma proteins as well as very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL).

It is known that HDL is inversely correlated with the severity of coronary arterial lesions as well as with the likelihood that these will progress. Therefore, removal of HDL is not advantageous.

Known aphaeresis techniques also exist which can remove LDL from plasma. These techniques include absorption of LDL in heparinagarose beads (affinity chromatography) or the use of immobilised LDL-antibodies. Other methods presently available for the removal of LDL involve cascade filtration absorption to immobilised dextran sulphate and LDL precipitation at low pH in the presence of heparin. Each method specifically removes LDL but not HDL.

LDL aphaeresis has, however, disadvantages. Significant amounts of other plasma proteins are removed during aphaeresis and to obtain a sustained reduction in LDL-cholesterol, LDL aphaeresis must be performed frequently (up to once weekly). Furthermore, LDL removal may be counter productive as low blood LDL levels may result in increased cellular cholesterol synthesis.

To satisfy the need for a method of achieving a reduction in plasma cholesterol in homozygous familial hypercholesterolemia, heterozygous familial hypercholesterolemia and patients with acquired hyperlipidaemia other than by diet, drug therapy, LDL

aphaeresis, or a combination of these, an extra corporeal lipid elimination process, termed "cholesterol aphaeresis", has been developed. In cholesterol aphaeresis, blood is withdrawn from a subject, plasma separated from the blood and mixed with a solvent mixture which extracts lipid from the plasma, after which the delipidated plasma is recombined with the blood cells and returned to the subject.

In more detail, cholesterol aphaeresis results in the removal of fats from plasma or serum. However, unlike LDL aphaeresis, the proteins that transport the fat (apolipoproteins) remain soluble in the treated plasma or serum. Thus the apolipoproteins of VLDL, LDL and HDL are present in the treated plasma or serum. These apolipoproteins, in particular apolipoproteins A1 from the defatted HDL in the plasma or serum, are responsible for the mobilisation of excessive amounts of deposited fats such as cholesterol in arteries, plaques, or excessive amounts of triglycerides, adipose tissue, or fat soluble toxins that are present in adipose tissue. These excessive amount of fats or toxins are transferred to the plasma or serum, bound to the newly assembled lipoproteins. Thus by applying another cholesterol aphaeresis procedure, these unwanted fats or toxins are successively removed from the plasma and thus the body.

The main advantage of this procedure is that LDL and HDL are thus not removed from the plasma but only cholesterol, some phospholipids and considerable triglycerides. United States Patent No 4,895,558 describes such a system.

While cholesterol aphaeresis has overcome the shortcomings of dietary and/or drug treatments and other aphaeretic techniques, existing apparatus for cholesterol aphaeresis does not provide a sufficiently rapid and safe process. For use in a clinical setting, apparatus is required which effects delipidation more efficiently. Furthermore, flow rates of the order of 70 ml/min are required for cholesterol aphaeresis of a human subject.

Thus the cholesterol aphaeresis described in the afore-mentioned US Patent No 4,895,558 was improved by incorporating into the system a spinner to disperse the incoming plasma laterally into the extracting solvent in the form of fine droplets to improve separation efficiency. This improved system is described in International Patent Application No PCT/AU94/00415.

Unfortunately, practice has established that the cholesterol aphaeresis systems described above still suffer from a number of disadvantages.

The first disadvantage is the explosive nature of the solvents used to delipidate this plasma. These solvents are, by the very nature of the continuous systems, in close proximity to the patient and medical staff. This hazard is clearly present for the duration of the delipidation process which usually runs for several hours.

The second disadvantage is that, in the prior continuous systems, a reliable procedure is not available to remove totally all of the solvents used in the delipidation before the treated plasma is returned to the patient.

In particular, the use of the preferred solvent 1-butanol in the delipidation is of concern as it can now be established that that solvent can be present as 1% to 5% of the treated plasma that is returned to the patient. This is because continuous systems can only include a single wash to remove solvents such as 1-butanol and a single wash is now found to be insufficient. It is not possible to provide sequential multi-washes in a continuous system because the patient would have to supply an unacceptable volume of blood to maintain each stage of the system overall and the patient would also be subjected to an increased hazard factor from the prolonged exposure to the solvents.

The long term toxicity of 1-butanol is not known, especially when directly present in the blood stream - it may cross the blood brain barrier. Certainly, external contact with this solvent is known to cause irritation of mucous membranes, contact dermatitis, headaches, dizziness and drowsiness.

A third disadvantage is that the continuous systems described above are not suitable for the delipidation of serum. If serum can be delipidated, there would be the advantage of favourably altering the blood rheology in that the viscosity will decrease following delipidation resulting in better haemodynamics for the originally impaired blood circulation.

Yet a fourth disadvantage is that delipidation in a continuous system is undertaken over several hours. Apart from the prolonged exposure to the hazardous solvents as discussed above, the equipment and staff are committed to a single patient. As the removal of

plasma or other blood fractions and their subsequent return to the patient as individual steps each only take a few minutes, it would be advantageous if the relatively lengthy delipidation step could be undertaken off site, thus freeing the patient, medical staff and equipment for other matters.

Finally, in a continuous system, clearly it is only the patient's own blood fraction that can be returned to that patient. However, for example, if the patient's plasma or serum could be removed and treated remote from the patient, then either autologous or non-autologous plasma or serum could be returned to the patient at a later date.

SUMMARY OF THE INVENTION

It is an object of the present invention to overcome, or at least ameliorate, the above-mentioned disadvantages in the provision of a method for delipidating not only plasma but also serum and other blood fractions which substantially reduces the exposure of the patient to the potentially hazardous solvents used, which also can effectively remove all traces of solvent(s) used in that delipidation, and which significantly reduces the contact time between the patient and the actual delipidation process.

It is a further object to provide a method whereby advantageous changes to the blood rheology of the originally impaired blood circulation of the patient can be achieved.

It is yet another object to provide a method whereby a patient's plasma or serum can be treated remote from that patient, thus allowing either autologous or

non-autologous plasma or serum to be returned to the patient at a later date.

In one aspect of the present invention, there is provided a method for the removal of cholesterol, triglycerides and other lipids from animal plasma, serum or other suitable blood fractions, as a discontinuous flow system, said method comprising withdrawing blood from a subject, separating the required fraction from the blood and mixing with a solvent mixture which extracts the said lipids from the fraction, after which the delipidated fraction is recombined with the blood cells and returned to the subject, characterised in that the solvent extraction step is carried out separately and remote from the subject.

Preferably, as part of the solvent extraction step, beads are used when mixing the blood fractions with the solvent. More preferably, the beads have a density substantially mid-way between the density of the fraction and the density of the solvent mixture. This ensures efficient mixing with a large surface area, increasing the efficiency of the extraction and also serving as a good separator of the plasma from the solvent when centrifugation is used to isolate the phases after extraction.

Preferably, to obtain a density substantially mid-way between the density of the fraction and the density of the solvent mixture, the beads contain entrapped air.

More preferably, as the density of plasma is approximately 1.006 g/ml and the solvents used generally have a density of approximately 0.8 g/ml, the density of the beads will be around 0.9 g/ml.

The beads may be manufactured from any acceptable material such as glass or plastic.

Once the resultant delipidated fraction-containing phase has been isolated, all traces of the extraction solvent must be removed before the fraction is recombined with the blood cells and/or returned to the subject.

One way of removing this solvent is to wash with another solvent, preferably diethyl ether, to remove substantially all of the original solvent used in the extraction step.

More preferably, four (4) washes are undertaken.

However, as another aspect of the present invention, efficient removal of the extraction solvent can be achieved by mixing the delipidated fraction with an absorbent specific for the solvent that is being removed.

In particular, the absorbent is contained in the pores of sintered spheres.

More preferably, the sintered spheres are approximately 2 to 5 mm in diameter with the pores of the spheres being less than 50 Å in diameter. Most preferably, the spheres are manufactured from glass.

Preferably, the absorbents used in the sintered spheres are the macroporous polymeric beads for absorbing organic molecules from aqueous solutions marketed by Bio-Rad Laboratories under the trade name Bio-Beads SM.

If the solvent used to delipidate the fraction is 1-butanol, then the absorbent is preferably Bio-Beads SM-2.

Preferably, the absorbent is held in a chamber which is adapted to allow the delipidated fraction to pass through or over the absorbent at least twice if a single pass is insufficient to remove all of the solvent.

Preferably, as part of isolating the delipidated fraction-containing phase, that phase is subsequently washed with another solvent, preferably diethyl ether, to remove a substantial amount of the original solvent before the treatment with the absorbent.

More preferably, that phase is washed at least three (3) times.

The plasma may be human plasma or plasma from other living animals. The plasma can be obtained from human or animal blood by known plasma separating techniques which include centrifugal separation, filtration and the like.

Similarly, the serum or other lipid-containing fraction can be derived from human or other living animals by known techniques.

Suitable solvents for the extraction comprise mixtures of hydrocarbons, ethers and alcohols. Preferred solvents are mixtures of lower alcohols with lower ethers. The lower alcohols suitably include those which are not appreciably miscible with the plasma and these can include the butanols (butan-1-ol and butan-2-ol). C_{1-4} ethers are also preferred and these can include the propyl ethers (di-isopropyl

ether and propyl ether). Other solvents which may be applicable include amines, esters, hydrocarbons and mixtures providing that the solvent can (1) rapidly and preferably remove cholesterol from the plasma, (2) is substantially immiscible with the plasma, (3) can be removed from the plasma, and (4) does not denature the desired moieties. Preferred solvent compositions are butanol with di-isopropyl ether and these may be in the ratio of 0% - 40% of the alcohol to 100% - 60% of the ether.

DETAILED DESCRIPTION OF EMBODIMENTS

Materials and Methods

Animals

The roosters used in this study were of White Leghorn Hiline strain and were obtained as one-day old chicks. All roosters from 8 weeks old were transferred into individual cages. Water and feed were supplied unrestricted. At eight weeks of age, 15 control birds were fed a commercial poultry ration for 31 days and another group of 30 birds were injected subcutaneously each day with 5mg diethylstilboestrol (DES) in sesame oil for a period of 31 days. In addition they were fed on the same commercial diet which was supplemented with 2.6% (w/w) cholesterol for a period of 31 days. Fifteen animals of the DES treated group were then subjected to lipid aphaeresis (LA). Fifteen animals of the DES treated group had sham treatments. Once the LA or sham treatments commenced, all animals were fed the standard poultry ration, except during the actual treatment itself when animals were kept off their feed for three hours following reinfusion of their

autologous blood. Animals were sacrificed two days following the 4th treatment, LA or sham.

Lipid Aphaeresis Procedure

Approximately 25% of the calculated blood volume was collected from a brachial vein of the animal with a 21 gauge needle and syringe. The total blood volume was estimated at 8 percent of the body weight. The blood was collected in heparinized tubes and immediately centrifuged at 900 g for 5 minutes at room temperature. The blood cells were suspended in an amount of saline equivalent to the plasma volume and were reinfused into the animal. The plasma was kept refrigerated for twelve hours and was then delipidated for 20 minutes with a mixture of butanol and di-isopropyl ether (DIPE), 25:75 (v/v), in a ratio of one volume of plasma to two volumes of butanol-DIPE mixture (organic phase). Inert plastic beads with a density of 0.9g/mL (1g) were added to the mixture. After extraction, the mixture was centrifuged at 900 g for 2 min to separate the plasma and organic phases. The organic phase (upper layer) was removed, free of plasma phase, by careful aspiration with a pasteur pipette under vacuum. Traces of butanol in the plasma phase were washed out with four volumes of diethyl ether (DEE) for 2 min by end-over-end rotation at 30 rpm. The mixture was then centrifuged at 900 g for 2 min to separate plasma and ether phases. The ether phase was subsequently removed by aspiration with a pasteur pipette. Residual ether was removed by evacuation with a water pump aspirator at 37°C. The plasma was then passed through a 5 mL column containing Bio-Beads SM-2.

This procedure yielded delipidated plasma. The delipidated plasma was re-mixed with the blood cells of a subsequent 25% blood collection which was then reinfused through a brachial vein back into the identical donor animals. The duration of the entire procedure, that is, removal of blood from the animal to reinfusion of treated blood back to the animal was approximately 1 hour. After the fourth lipid aphaeresis treatment, the animals were sacrificed and their livers and aortae were dissected. The LA treatment procedures were repeated 3 times after the first treatment.

Sham Treatment Procedures

This was essentially the same as the LA procedure with the exception of the plasma delipidation with the organic solvents. The blood was collected in heparinized tubes and immediately centrifuged at 900 g for 5 min. The plasma was separated from the blood cells. The blood cells were mixed with saline in the same volume of the collected plasma and reinfused into the animal. The plasma was kept refrigerated for twelve hours and was then remixed with blood cells of a subsequent 25% blood collection after the second and/or subsequent plasma separations. After the fourth lipid aphaeresis treatment, the animals were sacrificed and their livers and aortae were dissected. The sham treatment procedures were repeated 3 times after the first treatment.

Tissue Lipid Preparation

The livers were weighed, minced with a scalpel blade and homogenised in 0.9% sodium chloride solution by 10-12 strokes of a motor driven Teflon-glass

homogeniser (1900 rpm). The aorta was weighed and three times its weight of 3 mm glass beads were added in a homogenising bottle containing 0.9% sodium chloride. The contents were then homogenised for one minute. The lipid from the homogenised liver and aorta samples were extracted by the Folch procedure and weighed.

Table 1 Effect of LA and sham treatments on the total lipid concentrations in livers and aortae of hyperlipidaemic roosters.

	UNTREATED CONTROLS n = 15	TREATED FOUR APHAERESIS APPLICATIONS	
		SHAM n = 15	LA n = 15
LIVER[a]	3.65 ± 0.98	5.53 ± 1.50 [b]	3.72 ± 1.00 [b]
AORTA[a]	6.01 ± 0.97	8.11 ± 2.15 [c]	6.12 ± 0.95 [c]

[a] Total lipid concentrations expressed as g lipid per 100 g tissue, mean ± SD

[b, c] p values were < 0.05 when sham treatments were compared with LA treatments.

There were no statistical differences between the values of corresponding tissues in the untreated control group and the LA treated group.

All animals were sacrificed two days after the final aphaeresis treatment.

Humans

Patients have the plasmapheresis procedure undertaken using known transvenous techniques and plasmapheresis systems.

Plasmapheresis is performed using vein-to-vein or arteriovenous fistula in the forearm of patients. Heparin is given at the beginning of the procedure as a 5,000 unit bolus, and then by continuous infusion at the rate of 700 units per hour over the course of the procedure. Access through the antecubital veins should provide plasma flow rates of 25 to 40 mls per minute.

Blood taken from a patient is immediately treated with ACD-A (anticoagulant) in a ratio of between 1:8 and 1:16 (ACD-A:blood). The plasma is separated from this solution using a conventional plasmapheresis machine.

Twenty five percent plasma is removed from the patient. This represents one percent of the ideal body weight.

Only the first volume of plasma collection is replaced with plasma replacement fluid to the patient.

The plasma is kept refrigerated up until twelve hours prior to reinfusion of delipidated plasma in exchange for another twenty five percent plasma collection (weekly or biweekly).

The plasma is delipidated and the delipidated plasma is tested to ensure all solvent has been removed

before the clean delipidated plasma is exchanged for new untreated plasma.

In one embodiment of the present invention, the continuous flow system described in US Patent No 4,895,558 (the entire content of which is included herein) is modified to a discontinuous system by removing the appropriate blood volume to be treated and subjecting that volume to delipidation at a site remote from the patient.

In another embodiment of the present invention, the continuous flow system described in International Patent Application No PCT/AU94/00415 (the entire content of which is included herein) is modified to a discontinuous system by removing the appropriate blood volume to be a site remote from the patient before the plasma is dispersed into small droplets into the solvent by the dispersing means.

In either of the above embodiments, the extraction step can include, in accordance with the present invention, either multiple washing of the extracted phase and/or using an absorbent.

For example, the plasma is delipidated with a solvent mixture comprising 1-butanol and di-isopropyl ether. The delipidated fraction is then washed three (3) or four (4) times with diethyl ether. After the final wash, the diethyl ether is removed by centrifugation and vacuum extraction at 37°C. The sintered spheres containing Bio-Beads SM-2 are then mixed with the delipidated plasma to remove the final traces of 1-butanol.

Conclusions

DES administration to the roosters resulted in a significant amount of fat (lipid) accumulation in the livers and aortae.

Discontinuous LA treatments corresponding to approximately one plasma volume treated by four applications of 25% of plasma volume treated per time resulted in significant decreases in both hepatic and aortic lipids in hyperlipidaemic animals. Moreover, the LA treated hyperlipidaemic animals ended up with lipid values that were similar to control animals.

(i) These experiments show that excessive amounts of body fats in the form of adipose tissue (triglycerides) in the liver can be removed by LA; and

(ii) regression of atherosclerosis occurs in the aorta by LA treatments.

Similar results can be expected for human patients.

By adapting the prior art methods to discontinuous flow systems, the present invention can remove or at least significantly reduce any danger to patients and medical staff from the explosive nature of the solvents employed.

Further, by using the improved solvent extraction methods of the present invention, all of the potentially poisonous extraction solvents can be removed before the treated blood is returned to the patient.

Also, the improved solvent extraction method of the present invention is not limited to plasma delipidation but also it is applicable to the delipidation of serum, thus providing advantageous changes to the blood rheology of the originally impaired blood circulation of the patient.

The present invention thus provides for a rapid regression of coronary atherosclerosis in a patient.

Finally, as the present invention is a discontinuous system, it is not essential to return the delipidated blood fraction immediately to the patient. It is already known that plasma or serum can be collected and stored under sterile conditions in a refrigerator or freezer for extended periods and that it can be returned safely to the patient within twelve (12) hours of breaking the sterile seal. Therefore, if necessary, reintroduction of the delipidated fraction can occur several weeks after it was first removed from the patient. This option leads to particular advantages such as, economies of scale when several patients have to be treated simultaneously, the freeing of medical staff and equipment for other duties, and the reduction in stress for the patient whom no longer has to be hooked up to a delipidation apparatus for several continuous hours. Further, it enables a bank of plasma or serum to be maintained which is free of any infection which can be delipidated and exchanged for a patient's plasma or serum as required. Of course, autologous or non-autologous plasma or serum could be returned to the patient under these conditions.

The embodiments are described by way of illustrative examples only and various changes and modifications

may be made thereto without departing from the inventive concept as defined in the following claims.

CLAIMS:

1. A method for the removal of cholesterol, triglycerides and other lipids from animal plasma, serum or other suitable blood fractions, as a discontinuous flow system, said method comprising withdrawing blood from a subject, separating the required fraction from the blood and mixing with a solvent mixture which extracts the said lipids from the fraction, after which the delipidated fraction is recombined with the blood cells and returned to the subject, characterised in that the solvent extraction step is carried out separately and remote from the subject.

2. A method as defined in Claim 1, wherein the extraction solvent is substantially removed from the delipidated fraction by washing with a second solvent.

3. A method as defined in Claim 2, wherein the delipidated fraction is washed four times.

4. A method as defined in Claim 2 or Claim 3, wherein the second solvent is diethyl ether.

5. A method as defined in any one of Claims 1 to 4, wherein the solvent extraction step comprises:

(a) mixing the solvent mixture containing the fraction with beads, said beads being of a density substantially mid-way between the density of the fraction and the density of the solvent mixture; and

(b) isolating the thus delipidated fraction-containing phase.

6. A method as defined in Claim 5, wherein the beads contain entrapped air to obtain the density substantially midway between the density of the fraction and the density of the solvent mixture.

7. A method as defined in Claim 6, wherein the density of the beads is about 0.9 g/ml.

8. A method as defined in Claim 1, wherein the extraction solvent is removed from the delipidated fraction by mixing the delipidated fraction with an absorbent specific for the extraction solvent.

9. A method as defined in Claim 8, wherein the absorbent is contained in the pores of sintered spheres.

10. A method as defined in Claim 9, wherein the sintered spheres are about 2mm to 5mm in diameter and the pores of the spheres are less than Å in diameter.

11. A method as defined in any one of Claims 8 to 10, wherein the absorbent is a macroporous polymeric bead for absorbing organic molecules from an aqueous solution.

12. A method as defined in any one of Claims 8 to 11, wherein the absorbent is held in a chamber which is adapted to allow the delipidated fraction to pass through or over the absorbent at least twice.

13. A porous sintered sphere for use in a method as defined in any one of Claims 8 to 12, said sphere containing an absorbent in its pores.

14. A sintered sphere as defined in Claim 13, wherein the absorbent is a macroporous polymeric bead for absorbing organic molecules from an aqueous solution.

15. A method of changing the blood rheology of an animal with impaired blood circulation whereby the plasma, serum or other suitable blood fraction of the animal has been treated by a method as defined in any one of Claims 1 to 12.

16. A method for rapid regression of coronary atherosclerosis in an animal whereby the plasma, serum or other suitable blood fraction from the animal is treated by a method as defined in any one of Claims 1 to 12.

17. A method of removing excessive adipose tissue from an animal whereby the plasma, serum or other suitable blood fraction from the animal is treated by a method as defined in any one of Claims 1 to 12.

18. A method of removing fat soluble toxins from an animal whereby the plasma, serum or other suitable blood fraction from the animal is treated by a method as defined in any one of Claims 1 to 12.

19. A method of changing the blood rheology of an animal whereby the plasma or serum of the animal is exchanged for non-autologous plasma or serum wherein said non-autologous plasma or serum has

been treated by a method as defined in any one of Claims 1 to 12.

20. A method of rapidly regressing coronary atherosclerosis in an animal whereby the plasma or serum of the animal is exchanged for non-autologous plasma or serum wherein said non-autologous plasma or serum has been treated by a method as defined in any one of Claims 1 to 12.

21. A method of removing excessive adipose tissue from an animal whereby the plasma or serum of the animal is exchanged for non-autologous plasma or serum wherein said non-autologous plasma or serum has been treated by a method as defined in any one of Claims 1 to 12.

22. A method of removing fat soluble toxins from an animal whereby the plasma or serum of the animal is exchanged for non-autologous plasma or serum wherein said non-autologous plasma or serum has been treated by a method as defined in any one of Claims 1 to 12.

Attorney Docket No. 7676-46

Applicant or Patentee: Karim Rouan Cham

Serial or Patent No.: Not Yet Assigned
Filed or Issued: June 10, 1997
For: A Treatment For Cardiovascular And Related Diseases

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

[ ] the owner of the small business concern identified below.

[x] an official of the small business concern empowered to act on behalf of the concern identified below.

NAME OF CONCERN Aruba International Pty Ltd
ADDRESS OF CONCERN 14/1465 Ipswich Road, Rocklea, Queensland, 4106, Australia

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Sections 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled A TREATMENT FOR CARDIOVASCULAR AND RELATED DISEASES
by inventor(s) Karim Rouan CHAM

described in

[x] the specification filed herewith.
[ ] Application Serial No. _____
Filed _____
[ ] Patent No. _____
Issued _____

If the rights held by the small business concern are not exclusive, each individual concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME _____
ADDRESS _____
_____

[ ]Individual [ ]Small Business Concern [ ]Nonprofit Organization

FULL NAME _____
ADDRESS _____
_____

[ ]Individual [ ]Small Business Concern [ ]Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Bill Elliot CHAM
TITLE IN ORGANIZATION Director
ADDRESS OF PERSON SIGNING 353 Woodlands Drive, Sheldon, Queensland, 4157, Australia

28 May 1997
DATE

SIGNATURE

567.1

Attorney Docket No. 7676-46

DECLARATION AND POWER OF ATTORNEY
(Original Application)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled A TREATMENT FOR CARDIOVASCULAR AND RELATED DISEASES

the specification of which (check one)

[X] is attached hereto.

[ ] was filed on _____

as Application Serial No. _____

[X] was described and claimed in PCT Application No. PCT/AU95/00875 filed on 22 December 1995

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to herein.

I acknowledge the duty to disclose information which is material to patentability in accordance with Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119, of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

FOREIGN PRIORITY APPLICATION(S)

			Priority Claimed
PN0307	Australia	22 December 1994	[x] Yes [ ] No
(Number)	(Country)	(Day/month/ year filed)	
			[ ] Yes [ ] No
(Number)	(Country)	(Day/month year filed)	

And I hereby appoint Ronald L. Panitch, Registration No. 22,825; William W. Schwarze, Registration No. 25,918; Alan S. Nadel, Registration No. 27,363; Leslie L. Kasten, Jr., Registration No. 28,959; Joel S. Goldhammer, Registration No. 22,130; Wallace D. Newcomb, Registration No. 14,823; John Jamieson, Jr., Registration No. 29,546; Martin G. Belisario, Registration No. 32,886; Lynda L. Calderone, Registration No. 35,837; Charles E. Bergère, Registration No. 36,337; David W. Parker, Registration No. 37,414; Steven H. Meyer, Registration No. 37,189; Randolph J. Huis, Registration No. 34,626; and Clark A. Jablon, Registration No. 35,039, as my attorneys or agents with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Address all correspondence to PANITCH SCHWARZE JACOBS & NADEL, P.C., 1601 Market Street, 36th Floor, Philadelphia, Pennsylvania 19103. Please direct all communications and telephone calls to William W. Schwarze at 215-567-2020.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may

jeopardize the validity of the application or any patent issued thereon.

1-00

Full name of sole or first inventor Karim Rouan CHAM

Inventor's Signature _____

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_____

Full name of second joint inventor, if any _____

Inventor's Signature _____

Date _____

Residence _____

Citizenship _____

Post Office Address _____

_____

Full name of third joint inventor, if any _____

Inventor's Signature _____

Date _____

Residence _____

Citizenship _____

Post Office Address _____

_____